CMCU 01F07-05

- Protein Engineering and Bioactive Molecules Laboratory, LIP-MB INSAT,
- **Pr M. Nejib Marzouki**
- Cellular and Protein Engineering Laboratory, University of La Rochelle France, **Dr Thierry Maugard**.

-

Glycosides Hydrolyzing Enzymes analysis, process engineering to produce biotechnological substrates for cosmetic, food and therapeutic molecules

The filamentous fungus *Sclerotinia sclerotiorum* produces β-glucosisdases in liquid culture with a variety of carbon sources, including cellulose (filter paper), xylan, barley straw, oat meal, and xylose. Analysis by native polyacrylamides gel electrophoresis (PAGE) followed by an activity staining with the specific chromogenic substrate, 5-bromo 4-chloro 3-indolyl β-1,4 glucoside (X-glu) showed that two extracellular β-glucosidases, designated as β-glu1 and β-glu2,. Only one enzyme designated as β-glu x was revealed by the same method in the xylose culture filtrate.

β-glu1 and β-glu2 were purified to homogeneity. β-glu1 have a molecular mass of 196 kDa and 96.5 kDa, as estimated by gel filtration and sodium dodecyl sulfate (SDS)–PAGE respectively, suggesting that the native enzyme may consist of two identical subunits. β-glu2 is a monomeric protein with an apparent molecular mass of about 76.5 kDa. Also,β-glu1 and β-glu2 were biochemical and kinetic characterized.

The *β*-glucosisdases, an extracellular *β*-glucosisdase-X produced on xylose, and a commercial *β*-glucosidase from *Aspergillus niger*, were used to synthesize GOS from cellobiose. Yet, specially, *β*-(1-6) branched *β*-(1-3) gluco-oligosaccharides, corresponding to the structure of epiglucan. Gentiobiose, cellotriose, cellotetraose, *β*-Glc-(1-3)-*β*-Glc-(1-4)-Glc, *β*-Glc-(1-6)-*β*-Glc-(1-4)-Glc and *β*-Glc-(1-6)-*β*-Glc-(1-3)-Glc were synthesized from cellobiose by both enzymes. The latter compound was preferentially synthesized by the Sc-*β*- glycosidase-X. Under the best conditions. Only **7 g l–1** of *β*-Glc-(1-6)-*β*-Glc-(1-3)-Glc was synthesized by the An-*β*-glycosidase and **20 g l–1** synthesized with Sc-*β*-glycosidase-X respectively.

For the first time, we have performed the synthesis of the Epiglucan domain of GOS with pharmaceutical applications by an innovative and efficiently process carried outby the Sc-*β*-glycosidase-X.

Production of xylosidase and glucosidase by the fungus *Sclerotinia sclerotiorum* was optimized in the presence of different carbon sources. Immobilization supports with different physico-chemical characteristics were evaluated for use in continuous reactors process.

Immobilization and activity yields were analyzed on Duolite, Amberlite, Celite and DEAE-sepharose, and entrapment in polyacrylamide gel or reticulation using glutaraldehyde. The highest yields were obtained for *β*-xylosidase adsorbed on Duolite A7 and *β* glucosidase linked on DEAE-sepharose.

Enzyme preparations from *S. sclerotiorum* cultures were used in a biphasic (alcohol/aqueous) medium for the synthesis of alkyl-glycosides by *trans*-glycosylation of sugars and long-chain alcohols.

Highest yields were obtained with xylan and C4–C6-alcohols. The reaction produced alkyl-_-xyloside and alkyl-_-xylobioside, as confirmed by MS/MS. Up to 22mM *iso*-amyl-xyloside and 14mM *iso*-amyl-xylobioside.

Alkyl-glycosides offer potential industrial applications as non-ionic surfactants and are the topic of active research.